

## THE REVERSIBLE REPLACEMENT OF POTASSIUM BY RUBIDIUM IN *ULVA LACTUCA*<sup>1</sup>

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The substitution of rubidium ion for potassium ion in physiological processes has been studied for over 80 years. This subject is of special interest in view of the close chemical similarities of the two ions, rubidium being much more akin to potassium than is sodium. Permeability studies have revealed a high penetration rate of both rubidium and potassium ion (Brooks, 1932, 1939).

Studies on the replacement of potassium by rubidium have compared, on the one hand, the amount of physical replacement of potassium by rubidium within the cell, and on the other hand the suitability of rubidium as a substitute for potassium physiologically. Complete physical replacement of potassium by rubidium has been shown for *Chlorella* (Pirson, 1939), and yeast (Scott, unpublished data); partial physical replacement occurs in vertebrate muscle (Follis, 1943; Heppel and Schmidt, 1938; Mitchell, Wilson and Stanton, 1921) and in the duckweed *Lemna minor* (Pirson and Kellner, 1952). Rubidium can completely substitute for potassium in the repolarization of nerve (Feng and Liu, 1951; Gallego and De No, 1947), the restoration of heart beat in the frog heart (Ringer, 1884; Zwaardemaker, 1919), the activation of spermatozoa (White, 1953), the transport into the erythrocyte (Love and Burch, 1953), the activation of zymase (Giordani, 1932), the respiration of mitochondria (Pressman and Lardy, 1952), the bacterial production of pyruvic acid from malic acid (Lwoff and Ionesco, 1947), the activation of pyruvic phosphoferase (Kachmar and Boyer, 1953), and for growth in the bacterium *Streptococcus faecalis* (MacLeod and Snell, 1948). Rubidium is less effective than potassium in supporting assimilation, chlorophyll formation and cell division in *Chlorella* (Pirson, 1939), in supporting growth in *Lactobacillus casei* (MacLeod and Snell, 1948), or *Nitzschia closterium* (Stanberry, 1934), or for the secretion of adrenalin (Hermann, 1942). Rubidium is ineffective in supporting antibiotic activity in the subtilin-producing strain of *Bacillus subtilis*, although it promotes growth as well as does potassium in this bacterium (Feeney and Garibaldi, 1948). The element is definitely toxic at high concentration in certain bacteria, being antagonized by potassium (Scharer and Schropp, 1933), and is fatal to rats when it is substituted for up to 50-66 per cent of the potassium in the tissues (Follis, 1943; Heppel and Schmidt, 1938; Mitchell, Wilson and Stanton, 1921; Zipser and Freedberg, 1952).

Previous work has been concerned with the extent of replacement of potassium

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by rubidium or the physiological results thereof, rather than with the kinetics of replacement. The present work is a kinetic study of the reversible exchange of rubidium for potassium and of potassium for rubidium.

#### MATERIALS AND METHODS

The organism selected for this study was the green alga, *Ulva lactuca*. This marine organism, like most cells living in a high-sodium, low-potassium environment, normally accumulates potassium and partially excludes sodium. Consisting of large membranous fronds two cell layers in thickness, thus presenting a large surface area for interchange with the environment, this alga is particularly well suited for investigation of this nature.

Large fronds were collected from Perch Pond, near Falmouth, Mass., and conditioned for at least 24 hours in running sea water under incandescent illumination. Samples, cut from the same frond, were given a brief rinse in isotonic sucrose followed by a blot in absorbent toweling to remove most of the adherent sea water, then placed in large finger bowls with about 160 ml. per sample of an artificial sea water (Marine Biological Laboratory, Formulae and Methods IV, 1956), containing rubidium instead of potassium. The rubidium sea water was replaced with fresh rubidium sea water after 50 hours. Diffuse illumination was present during the experiment, which resulted in a slight drop in rubidium sea water pH. After 96 hours in rubidium sea water, the samples were placed in running sea water for 120 hours. Samples were removed in triplicate at various times, rinsed in isotonic sucrose for 30 seconds and blotted three times in absorbent tissue to remove extra-cellular sodium, potassium and rubidium ion. Wet and dry weights were taken and cell water calculated by difference. The samples were ground, extracted with 1 N HNO<sub>3</sub> for two hours at 110° C., and the extracts diluted to volume in 50-ml. volumetric flasks. The extracts were analyzed for sodium, potassium and rubidium ions by the Beckman flame spectrophotometer.

#### RESULTS

The initial uptake of rubidium is both rapid and nearly complete within the first four hours, the time at which the first samples were taken. At 96 hours the rubidium ion concentration reaches a maximum of about 87 per cent of the control value of potassium; longer immersion in rubidium sea water does not result in an increase above this maximal concentration value. The potassium loss is initially rapid and then continues to decrease in a manner parallel to that of the control, reaching a minimum of about 13 per cent of the control (Fig. 1).

When the fronds are placed in running sea water, the kinetics of the replacement of rubidium by potassium are quite different from those involving the replacement of potassium by rubidium. After an initial sharp drop in rubidium for 10 hours and a sharp rise in potassium for 10 hours, the rates of loss of rubidium and rise of potassium decrease. After 120 hours in running sea water the potassium content of the alga has practically reached that of the control, whereas the rubidium content has been reduced to only 30 per cent of its maximal value. Longer immersions were not practical, as the alga begins to show signs of aging.

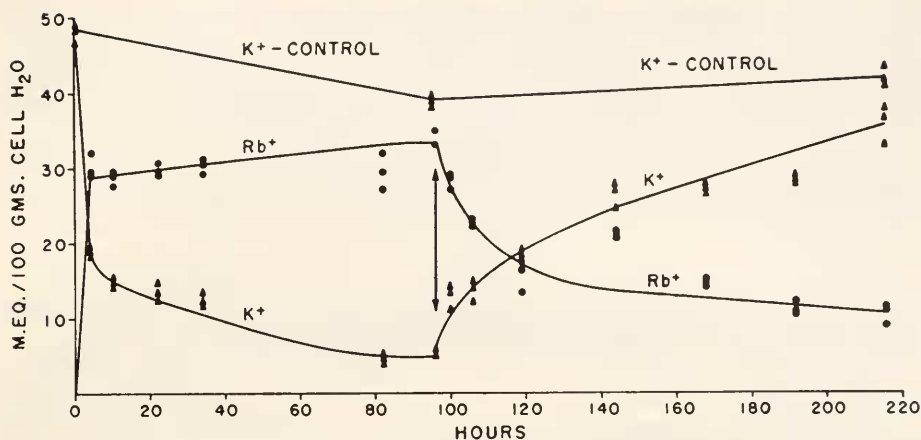


FIGURE 1. The replacement of potassium by rubidium within *Ulva lactuca* in rubidium sea water, and the replacement of rubidium by potassium on transfer of the alga to running sea water. The arrow indicates the time of transfer to running sea water. Concentrations are expressed on a cell water basis.

Throughout the experiment the sodium concentration of the samples remained essentially constant at  $26 \pm 4$  meq. per 100 gm. cell water.

#### DISCUSSION

The initial uptake of rubidium is too rapid to be explained on the basis of a new, separate uptake mechanism for rubidium alone, for such a mechanism would have to be much faster than that previously demonstrated for potassium in this alga (Scott and Hayward, 1954b). Rather, the rate of rubidium uptake is entirely consistent with the known rate of potassium turnover at  $20^\circ$  (Scott and Hayward, 1954a). Therefore, rubidium is being transported by the same mechanism as is potassium. The cessation of rubidium uptake is probably the result of the establishment of an equilibrium between the rubidium and the potassium concentrations, for the total alkali metal base ( $\text{Na}^+ + \text{K}^+ + \text{Rb}^+$ ) is constant within  $\pm 5$  meq. of that of the control (76–65 meq./100 gm. cell water).

Rubidium does not seem to affect the final level of potassium re-accumulation, but it would appear to have an effect on the kinetics of potassium re-accumulation. The re-accumulation of potassium is too slow to be accounted for solely on the basis of exchange of potassium ion for rubidium ion. Rather, it would seem that two factors might be operative: 1) the full maintenance of the potassium-accumulation mechanism is not supported by rubidium ion; 2) the age of the samples may be such as to render the postassium-accumulating mechanism sluggish. Since *Ulva* cycles from gametophyte to sporophyte generation on the order of every two weeks, it will be seen that the time course of the experiment (9 days) includes most of one phase of the cycle of the organism. (Whether a particular frond was gametophyte or sporophyte was not determined in this work, for the two generations are morphologically identical.)

In another experiment samples were left in rubidium sea water for as long as 144 hours before transfer to running sea water. The rate of potassium re-accumulation was identical to that found after 96 hours in the experiment reported above; hence the increased time in rubidium sea water was not progressively detrimental to the potassium-accumulating mechanism.

The presence of rubidium within this organism did not prevent the formation and discharge of a germinal ridge in some samples. These samples were not used in the analyses.

#### SUMMARY

1. Rubidium ion replaces two-thirds of the potassium of *Ulva lactuca* within four hours after being placed in rubidium-containing sea water.
2. The rubidium concentration does not increase more than 5 meq. during the remainder of the experiment.
3. Potassium re-accumulation in running sea water is slower than the initial exchange of rubidium ion for potassium ion.
4. The disparity in the exchange kinetics is discussed.

#### LITERATURE CITED

- BROOKS, S. C., 1932. The rate of penetration of rubidium into living cells of *Valonia* and its apparent ionic radii. *J. Cell. Comp. Physiol.*, **2**: 223-231.
- BROOKS, S. C., 1939. Ion exchanges in accumulation and loss of certain ions by the living protoplasm of *Nitella*. *J. Cell. Comp. Physiol.*, **14**: 383-401.
- FEENEY, R. E., AND J. A. GARIBALDI, 1948. Studies on the mineral nutrition of the subtilin-producing strain of *Bacillus subtilis*. *Arch. Biochem.*, **17**: 447-458.
- FENG, T. P., AND Y. M. LIU, 1951. The membrane potential of potassium depleted nerve. *Chinese J. Physiol.*, **18**: 61-70.
- FOLLIS, R. M., JR., 1943. Histological effects in rats resulting from adding rubidium or cesium to a diet deficient in potassium. *Amer. J. Physiol.*, **138**: 246-250.
- GALLEGO, A., AND R. L. DE NO, 1947. The effect of several univalent ions on frog nerve. *J. Cell. Comp. Physiol.*, **29**: 189-206.
- GIORDANI, M., 1932. Activation of zymase with rubidium chloride. *Ann. Chim. Applicata*, **22**: 153-156.
- HEPPEL, L. A., AND C. L. A. SCHMIDT, 1938. Studies on the potassium metabolism of the rat during pregnancy, lactation, and growth. *Univ. Calif. Pub. Physiol.*, **8**: 189-205.
- HERMANN, H., 1942. The effect of the cations of alkalies and alkaline earths on the secretion of adrenaline. *Bull. Acad. Med.*, **126**: 234-236.
- KACHMAR, J. F., AND P. D. BOYER, 1953. Kinetic analysis of enzyme reactions: the potassium activation and calcium inhibition of pyruvic phosphoferase. *J. Biol. Chem.*, **200**: 669-682.
- LOVE, W. D., AND G. E. BURCH, 1953. A comparison of potassium<sup>42</sup>, rubidium<sup>86</sup> and cesium<sup>134</sup> as tracers of potassium in the study of cation metabolism of human erythrocytes *in vitro*. *J. Lab. Clin. Med.*, **41**: 351-362.
- LWOFF, A., AND H. IONESCO, 1947. Replacement of potassium by rubidium and cesium in the bacterial production of pyruvic acid from malic acid. *C. R. Acad. Sci.*, **225**: 77-79.
- MACLEOD, R. A., AND E. E. SNELL, 1948. The effect of related ions on the potassium requirement of lactic acid bacteria. *J. Biol. Chem.*, **176**: 39-52.
- MARINE BIOLOGICAL LABORATORY, 1956. Formulae and Methods IV, p. 55.
- MITCHELL, P. H., J. W. WILSON AND R. E. STANTON, 1921. The selective absorption of potassium by animal cells. II. The cause of potassium selection as indicated by the absorption of rubidium and cesium. *J. Gen. Physiol.*, **4**: 141-148.
- PIRSON, A., 1939. The effect of alkali ions upon the growth and metabolism of *Chlorella*. *Planta*, **29**: 231-261.

- PIRSON, A., AND D. KELLNER, 1952. Physiological action of rubidium. *Ber. deut. botan. Ges.*, **65**: 276-286.
- PRESSMAN, B. C., AND H. A. LARDY, 1952. Influence of potassium and other alkali cations on respiration of mitochondria. *J. Biol. Chem.*, **197**: 547-556.
- RINGER, S., 1884. An investigation regarding the action of rubidium and cesium salts compared with the action of potassium salts on the ventricle of the frog's heart. *J. Physiol.*, **4**: 370-379.
- SCHARRER, K., AND W. SCHROPP, 1933. Sand and water culture experiments with lithium and rubidium with special reference to the possibility of substituting these elements for potassium in plant nutrition. *Ernähr. Pflanze*, **29**: 413-425.
- SCOTT, G. T., AND H. R. HAYWARD, 1954a. The influence of temperature and illumination on the exchange of potassium ion in *Ulva lactuca*. *Biochim. Biophys. Acta*, **12**: 401.
- SCOTT, G. T., AND H. R. HAYWARD, 1954b. Evidence for the presence of separate mechanisms regulating potassium and sodium distribution in *Ulva lactuca*. *J. Gen. Physiol.*, **37**: 601.
- STANBERRY, F. A., 1934. The replacement of potassium by rubidium in *Nitzschia closterium*. *J. Mar. Biol. Assoc.*, **19**: 931-940.
- WHITE, I. G., 1953. The alkali metal requirements of ram and bull spermatozoa. *Australian J. Biol. Sci.*, **6**: 716-724.
- ZIPSER, A., AND A. S. FREEDBERG, 1952. The distribution of administered radioactive rubidium<sup>86</sup> in normal and neoplastic tissue of mice and humans. *Cancer Res.*, **12**: 867-870.
- ZWAARDEMAKER, H., 1919. On physiological radioactivity. *J. Physiol.*, **53**: 273-289.